

## Autologous Cultured Fibroblasts: A Protein Repair System

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Cultured autologous fibroblasts create a living injectable system that has been utilized effectively to treat rhytids, depressed scars, subcutaneous atrophy, acne irregularities, and laser wounds. Autologous cultured fibroblasts (Isolagen) as a protein repair system is produced from a 3-mm cutaneous punch biopsy. Skin biopsies are initiated in an *in vitro* tissue culture system. The cells are expanded to produce large quantities of fibroblasts and extracellular matrix totaling 1.0 to 1.5 ml. This cellular system is injected into the patient after 8 weeks of cellular expansion. Each area of treatment is injected once every 2 weeks for three injections. A total of 1,450 patients in the United States and Europe have been treated at designated centers. A total of 4,800 injections were given. At the University of Medicine and Dentistry of New Jersey and Hackensack University Medical Center, 94 patients were treated from 1995 through 1999. Long-term follow-up ranged from 36 to 48 months. A subjective patient satisfaction survey showed 92% of the patients were satisfied with the grade of correction. A long-term follow-up survey revealed continuing improvement beyond the initial correction in 70% of patients. Results from other designated centers correlated with the authors' findings. Cultured autologous fibroblasts (Isolagen) appears to be a living, cellular, dynamic filler system capable of immediate correction and continued repair of dermal and superficial subcutaneous deficiencies.

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Autologous cultured fibroblasts (Isolagen) have been utilized successfully as a living, dynamic protein repair system for dermal and subcutaneous deficiencies since 1995. Cutaneous contour defects have been treated with injectable bovine collagen since its introduction in 1977.<sup>1</sup> Along with the bovine collagen, several other filling substances are described in the literature. These can be divided into either dermal or subcutaneous fillers. The dermal fillers, such as bovine

collagen, Fibril, and cross-linked human collagen, are acellular and usually resorb within 3 to 6 months. Gore-Tex, AlloDerm, fat and deepithelialized dermis require surgical insertion into the subcutaneous layer and do not treat dermal deficiencies. Purified bovine collagen is available as Zyderm I (35 mg per milliliter), Zyderm II (65 mg per milliliter), and Zyplast (collagen cross-linked with glutaraldehyde). One percent to 6% of patients receiving collagen injection may develop a localized hypersensitivity reaction.<sup>2,3</sup> Usually this presents as temporary erythema, pruritus, induration, and swelling. Overhalt and colleagues<sup>4</sup> described granulomatous foreign body reaction at the injection site and stated that this may be an allergic response to bovine antigen. Zyderm I is resorbed rapidly by tissue collagenases within several weeks. The higher concentration of collagen in Zyderm II resorbs within 3 months.<sup>5</sup> Each of the soft-tissue augmentation materials stated earlier has its own shortcomings. The subcutaneous synthetic materials require surgical insertion, and can be complicated by rejection, infection, palpability, and lack of natural appearance with dynamic movement. Even autologous fat injections have limitations, such as overcorrection, prolonged swelling, and inability to treat the dermis. Furthermore, surgical harvesting and processing is required before fat injection. Fat injection is technique dependent, and reapplication is often necessary because of its tendency to undergo resorption.<sup>6</sup>

Using autologous fibroblasts as an injectable system to repair dermal and subcutaneous defects such as rhytids and scars opens a new window for reconstructive surgeons. This system is made available by Isolagen Technologies (Paramus, NJ) and utilizes the patient's own fibroblast protein repair system, which can produce collagen locally and correct the contour deformity. This system can restore a patient's dermis that

has had a reduction of fibroblasts and collagen integrity as a result of photodamage, chronological aging, scarring, trauma, acne, or infection. The system can also be stored indefinitely in liquid nitrogen and provides a virtually limitless source of unaging fibroblasts.

## Methods

This process was developed by the lead author, and clinical trials were begun in 1995. Both pre- and posttreatment photographs were obtained along with specialized informed consent. The autologous fibroblast culture process was started with a 3-mm retroauricular punch biopsy. The surgical specimen was placed in a special transport media tube on ice and in a thermos. The donor site was closed with one buried absorbable suture and the specimen was shipped by overnight mail to the laboratory, where the cells were expanded by proprietary tissue culture techniques. Six weeks after the biopsy, patients were given a 0.1-cc test injection at the wrist level with a 30-gauge needle. The patient was instructed to record any discomfort or signs of allergic reaction.

Eight weeks after the biopsy, a 1.2-ml sterile vial containing expanded autologous cultured fibroblasts (Isolagen) was shipped back to the treating physician on ice and in a thermos. The injection was always scheduled within 24 hours of shipment to ensure optimal viability. Visibility studies showed that within 24 hours of shipping, viability was 95%. Viability dropped to 85% 48 hours postshipment, and dropped to 65% at 72 hours. Spectrophotometric analysis and gel electrophoresis showed that the Isolagen injectate did not contain any foreign proteins. The injection technique requires multiple passes throughout the dermis with the bevel of the needle pointed downward. It is necessary to fill in the skin defect with the injectate and also to attempt to create blanching and raise a wheal on the skin surface during the injection process. The Isolagen was injected with a 30-gauge needle into the superficial, middle, and deep dermal junctions, and into the scar tissue. Emla cream, nerve block, or plain ice application was used for anesthesia at the injection site.

**Table 1. Treatment Areas**

Treatment Area	N
Nasolabial folds	36
Glabellar areas	7
Lips and perioral	29
Acne scars	10
Cheeks	5
Lower lids	1
Jowl lines	1
Other scars	5

Isolagen has also been utilized as a pre- or postlaser treatment, as an adjunct to botulinum toxin, and to prolong the result of bovine collagen indefinitely. To gain a filler effect for early patient satisfaction and to treat difficult areas such as nasolabial folds, marionette lines, and glabellar furrows, three injections of Isolagen were used. Injections were spaced 2 weeks apart.

All patients were advised to take 1,500 mg ascorbic acid orally and to use either a glycolic or an ascorbic acid topical cream. Any patients with chronic diseases, disseminated or cutaneous cancers, autoimmune disorders, immune deficiency syndromes, skin conditions such as psoriasis, and undergoing corticosteroid therapy were eliminated from the study. All patients were followed on a weekly and then monthly basis. Results were evaluated on photographic analysis and patient outcome surveys.

## Patients and Results

A total of 1,450 patients were treated in the United States and Europe. The patients received a total of 4,800 injections from 1995 through 1999. At our clinic, 94 patients were treated. Conditions such as perioral and facial rhytids, nasolabial folds, glabellar furrows, lip vermillion, and scars (acne and traumatic) were treated (Table 1). Improvements were evaluated with pre- and postinjection photographs at 3 months and 6-month intervals thereafter. A subjective patient satisfaction survey was also utilized to evaluate results. The patients were asked to grade correction on a 10-point scale. The patients were also asked to grade their satisfaction of the process on a 10-point scale. From our study group, only 1 patient reported mild erythema at the injection site, which resolved in 2 to 3 days. The national

**Table 2. Long-Term and Short-Term Follow-up Results**

Parameter	No. of Patients	Short-Term Follow-up, 12 mo	Long-Term Follow-up, 36-48 mo
Patient satisfaction	94	92%	70%
Grading of correction	56	7.8	6.46

study group reported 13 patient reactions to the injection (0.27%). Of these, 11 were mild reactions with redness and swelling that resolved within 48 to 72 hours. Two patients reported moderate reactions with swelling and erythema for 7 to 10 days.

At 12 months the patients' average grading of their degree of correction was 7.8 points. At 12 months, 92% of the patients reported that they were pleased with the results. There were no reports of infection, rejection, granuloma formation, keloid formation, or overcorrection of the treated areas. Long-term follow-up was between 36 and 48 months. A long-term follow-up questionnaire revealed that 70% of the patients reported being satisfied with their long-term results (Table 2). Eighty-eight percent of this group of patients noted continuing improvement of their correction for periods up to 24 months.

## Discussion

The ideal filler would be an autologous, injectable material that provides long-term results, requires minimal surgery and tissue removal for initial tissue harvest, and has unlimited yield without the need for additional tissue harvest. Autologous cultured fibroblasts (Isolagen) satisfies all these criteria. Autologous cultured and expanded living fibroblasts create a protein repair system that appears to show continuing correction (improvement) for many months to years (Fig 1). These living cultured fibroblasts with an extracellular matrix restore the dermal collagen that has had a reduction resulting from photo-damage, aging, or scarring (Fig 2). Additionally, when the initial biopsy specimen has been obtained, the fibroblasts can be expanded repeatedly without the need for an additional biopsy. The autologous cultured fibroblast system can be

stored indefinitely in liquid nitrogen and can be renewed at any time in the future.

The only other autologous injectable material is a dispersion of nonviable, intact autologous collagen fibers that have been derived from the patient's dermal layer, which is removed during extensive surgical procedures. Therefore, this process produces very limited quantities of material. One-year results from a study by Devore and colleagues<sup>7</sup> indicate more than 75% correction after at least three injections. Unfortunately this application has limited yield. To obtain 1 cc of dispersed collagen fibers, as much as 40 cm<sup>2</sup> of resected skin is required. Repeated applications to achieve full correction necessitate additional skin excisions, and because this is an acellular system, collagenases will eventually cause dissolution of the results.

Bovine collagen injections are very short lived, and may cause localized hypersensitivity reactions, scarring, and nodularity.

Other centers have also studied Isolagen and have reported high levels of satisfaction and correction. Watson and associates<sup>8</sup> treated 10 adult patients for improvement of facial rhytids or depressed facial scars. They reported subjective improvement scores of 60% to 100% by patients, and similar scores by clinicians. Their subjective scores correlated directly with objective measurements by optical profilometry of silicone molds taken from each patient before treatment and 6 months after Isolagen therapy.

Chernoff (unpublished data) studied 104 consecutive patients (90 women, 14 men) for 18 months. His population received an average of three Isolagen injections to static rhytids, acne/pox scars, and nasolabial folds. Patient satisfaction in improving facial rhytids ranged from 40% to 60%; for scars, 60% to 75%; and for lips; 30% to 50% with an average time of 6 to 8 months to see the improvements (G. Chernoff, unpublished data).

Autologous cultured fibroblasts present a versatile system that can be used in conjunction with other procedures such as laser resurfacing to enhance correction. Chernoff (unpublished data) studied 50 patients (40 women and 10 men) who were divided into two groups. The first group received only the 1,320-nm Nd:YAG laser (Cool-



Fig 1. (A) Pretreatment view of perioral rhytids. (B) Four years posttreatment with Isolagen.



Fig 2. (A) Pretreatment view of left-cheek scar. (B) Three years posttreatment (three injections). (C) Histological analysis of the test injection site shows neocollagenesis (electron micrograph, original magnification  $\times 20,000$  before 71% reduction).



Fig 3. (A) Pretreatment view of perioral rhytids and marionette lines. (B) Three years postinjection with Isolagen and three 1-J passes with the Erbium laser.



Fig 4. (A) Pretreatment view of acne scars, nasolabial folds, lips. (B) View 3.5 years after autologous cultured fibroblast injection and a low-fluence, single-pass carbon dioxide laser treatment.



Fig 5. (A) Six-month-old nonhealing laser burn after laser resurfacing. (B) View 6 weeks after Isolagen injection (courtesy of G. Chernoff, MD).

Touch: Laser Aesthetics Inc., San Diego, CA), and the second group was pretreated with Isolagen and Cool-Touch (G. Chernoff, unpublished data). The average time to patient satisfaction in the control population was 6.5 months in women and 7 months in men, whereas the Isolagen- and Cool-Touch-treated population showed remarkably early satisfaction of 3.6 months in women and 4 months in men (Fig 1). We have shown a dramatic response to Erb-Nd:YAG laser resurfacing with only three passes at 1 J to correct a Baker grade III to IV perioral rhytid to a grade 0 to I (Fig 3). Pretreatment with Isolagen also enhanced laser resurfacing of acne scars and nasolabial folds (Fig 4).

Theoretically, a dermis rich in autologous fibroblasts should produce a better laser result or should require less aggressive laser treatments because of the healing response that can be generated by the additional fibroblasts.

Kuo and coworkers<sup>9</sup> showed in a porcine model that after cutaneous laser surfacing, new collagen formation is increased. Reports by oth-

ers confirm similar findings in their in vivo human studies: that new collagen is produced as long as 12 months after carbon dioxide laser resurfacing.<sup>10-12</sup> What better environment to produce collagen than one that has a dermis rich in fibroblasts?

Moy et al.<sup>13</sup> showed glycolic acid application caused a 10-fold upgrade in the production of procollagen in human fibroblast culture. Therefore, dermal pretreatment with autologous cultured fibroblast might also produce a better response to topical ascorbic acids and glycolic acids.

Another use of autologous cultured fibroblasts was discovered by Chernoff (unpublished data). He was able to treat laser burn wounds that had not healed for 3 to 9 months, in a period of 6 to 8 weeks after Isolagen injections (Fig 5; G. Chernoff and colleagues, unpublished data).

## Conclusion

Cultured autologous fibroblasts (Isolagen) are the first attempt at cellular therapy in the field of plastic and reconstructive surgery. When injected, Isolagen provides an immediate as well as a gradual, continual correction of dermal defects and facial rhytids, without evidence of resorption or loss of correction. A high patient satisfaction rating was obtained with the Isolagen process. Cultured autologous fibroblasts also can serve as a valuable adjunct to lasers, bovine collagen, chemical peels, and facial peels.

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## Open Discussion

Hakan Usal, MD

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*Raymond M. Dunn, MD (Worcester, MA):* I have two questions, Dr Usal. One relates to cost. I wonder if you would comment on a cost comparison between collagen and Isolagen. In addition, besides the cultured fibroblasts, what is the injection mix? Is it also a substantial proportion of autologous collagen that has been produced by these fibroblasts or is it purely a suspension of fibroblasts?

*Dr Usal:* It's purely fibroblasts. They are spun

and cloned in the laboratory and then sent back to the physician as a 1.2-mm vial. This is injected back into the patient.

*Dr Dunn:* It is suspended in what sort of solution?

*Dr Usal:* A special medium that maintains the viability. The present cost is about \$370, but when it goes into mass production, we hope it will come down in price.

*Gregory E. Rauscher, MD (Hackensack, NJ):* I just want to make a comment. I have found this material to be very helpful, particularly in the patient who has "dents" in the nose where there are some V-shape deformities. Rather than going through a major operation, with a single injection you can smooth out an incredible number of mishaps that might be coming your way in referral. Patients certainly prefer that to an operation.

*Dr Dunn:* One final question. Have you done any animal studies to demonstrate the actual production of collagen, which is presumably playing a role postinjection with Isolagen?

*Dr Usal:* We didn't do any studies on production of collagen in our group. However, the dermatological literature states that collagen is increased. We also combined this procedure with some laser procedures. After laser treatment, the collagen production is also increased, but we did not do a specific study. The only study we have done is of histological sections of the preinjected and injected sites. They showed that the collagen in the specimen was 0.2  $\mu\text{m}$  preinjection and 2.1  $\mu\text{m}$  after injection.

*Dr Dunn:* So although you are proposing continued viability of the fibroblasts, you don't know whether they are actually producing collagen locally?

*Dr Usal:* That is correct.